ABSTRACT

MANGANESE (II) SULFATE MONOHYDRATE

CAS No. 10034-96-5

Chemical Formula: $MnSO_4H_2O$ Molecular Weight: 168.95

Synonyms: Manganese sulfate; manganous sulfate; sulfuric acid, manganese^{2+} salt (1:1), monohydrate

Manganese is the 12th most abundant element in the earth's crust. The base metal does not occur naturally, but is a component of more than 100 minerals, including sulfides, oxides, carbonates, silicates, phosphates, and borates. In addition to occurring in foods and drinking water, manganese occurs in the atmosphere from dust, volcanic activity, forest fires, and industrial emissions. Manganese (II) sulfate monohydrate was chosen for study because of its stability, solubility, and availability. Toxicology and carcinogenesis studies were conducted by administering manganese (II) sulfate monohydrate (97% pure) in feed to groups of male and female F344/N rats and B6C3F$_1$ mice for 14 days, 13 weeks, and 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, germ cells of *Drosophila melanogaster*, and cultured Chinese hamster ovary cells.

14-DAY STUDY IN RATS

Groups of five male and five female rats received diets containing 0, 3,130, 6,250, 12,500, 25,000, or 50,000 ppm manganese (II) sulfate monohydrate. All rats survived to the end of the study. Male rats exposed to 50,000 ppm had a mean body weight gain 57% lower and a final mean body weight 13% lower than those of the controls. The mean body weight gain of 50,000 ppm females was 20% lower and the final mean body weight was 7% lower than those of the controls. During the second week, 50,000 ppm males and females exhibited diarrhea.

14-DAY STUDY IN MICE

Groups of five male and five female mice received diets containing 0, 3,130, 6,250, 12,500, 25,000, or 50,000 ppm manganese (II) sulfate monohydrate. One female mouse in the 25,000 ppm group died on day 1 of unknown causes; all other mice survived to the end of the study. Differences in body weights between exposed and control mice could not be attributed to chemical administration.

13-WEEK STUDY IN RATS

Groups of 10 male and 10 female rats received diets containing 0, 1,600, 3,130, 6,250, 12,500, or 25,000 ppm manganese (II) sulfate monohydrate. Mean daily ingestion of manganese (II) sulfate monohydrate ranged from 110 to 1,700 mg/kg body weight in males and 115 to 2,000 mg/kg in females. All rats survived to the end of the study. Mean body weight gains were marginally lower than that of controls in males exposed to 3,130 ppm or more; mean body weight gains were significantly lower than that of the controls in females exposed to 6,250, 12,500, or 25,000 ppm. At the end of the study, absolute and relative liver weights of all exposed male rats and of 25,000 ppm female rats were significantly lower than those of controls. The total leukocyte count in males was similar between exposed and control rats; however, neutrophil counts of all exposed groups were greater than those of the controls, whereas lymphocyte counts of the 6,250, 12,500, and 25,000 ppm groups were significantly lower than those of the controls. Total leukocyte counts in 6,250, 12,500, and 25,000 ppm females were significantly decreased because of a decrease in lymphocytes. Male rats also demonstrated marginal but significant increases in percent hematocrit and erythrocyte count in the 6,250, 12,500, and 25,000 ppm groups. No clinical or histopathologic findings in rats were chemical related.

13-WEEK STUDY IN MICE

Groups of 10 male and 10 female mice received diets containing 0, 3,130, 6,250, 12,500, 25,000, or 50,000 ppm manganese (II) sulfate monohydrate. Mean daily ingestion of manganese (II) sulfate monohydrate ranged from 330 to 7,400 mg/kg body weight in males and 390 to 6,900 mg/kg body weight in females. No deaths were chemical related. The mean body weight gains of exposed male mice and of 50,000 ppm female mice were significantly lower than those of controls. The absolute and relative liver weights of 50,000 ppm males were significantly lower than those of controls. The percent hematocrit and hemoglobin concentration of males and females exposed to 50,000 ppm were lower than those of the controls, and the mean erythrocyte volumes were significantly lower than those of the controls. The total leukocyte counts of males in the 25,000 and 50,000 ppm groups were significantly lower than that of the controls. No clinical findings were attributed to manganese (II) sulfate monohydrate ingestion. Epithelial hyperplasia and hyperkeratosis of the forestomach occurred in three 50,000 ppm males.

2-YEAR STUDY IN RATS

Groups of 70 male and 70 female rats were fed diets containing 0, 1,500, 5,000, or 15,000 ppm manganese (II) sulfate monohydrate. Based on average daily feed consumption, these doses resulted in the daily ingestion of 60, 200, or 615 mg/kg body weight (males) or 70, 230, or 715 mg/kg (females). Eight to 10 rats from each group were evaluated at 9 and 15 months.

Survival, Body Weights, Feed Consumption, and Clinical Findings

Survival of 15,000 ppm male rats in the 2-year study was significantly lower than that of the control group. The deaths of males in the control and exposure groups were attributed to a variety of spontaneous neoplastic and nonneoplastic lesions; however, the greater number of deaths in the 15,000 ppm group resulted from increased incidences of advanced renal disease related to ingestion of manganese (II) sulfate monohydrate. The decreased survival of the 15,000 ppm males did not occur until approximately week 93 of the study; before week 93, survival was similar in all groups. Survival of exposed females was similar to that of the controls. The mean body weight of 15,000 ppm male rats was within 5% of the control group until week 89; by week 104, the mean body weight of 15,000 ppm males was 10% lower than that of the control group. The mean body weights of 1,500 and 5,000 ppm male rats and all exposed female groups were similar to those of the controls throughout the study. Feed consumption by all exposure groups was similar to that by the control groups. No clinical findings were attributed to manganese (II) sulfate monohydrate ingestion.

Hematology, Clinical Chemistry, and Tissue Metal Concentration Analyses

No differences in hematology and clinical chemistry parameters attributable to the ingestion of manganese (II) sulfate monohydrate occurred between exposed and control groups. At both the 9- and 15-month interim evaluations, tissue concentrations of manganese were significantly elevated in the livers of 5,000 and 15,000 ppm male and female rats, with an accompanying depression of hepatic iron.

Pathology Findings

The ingestion of diets containing 15,000 ppm manganese (II) sulfate monohydrate was associated with a marginal increase in the average severity of nephropathy in male rats (0 ppm, 2.9; 1,500 ppm, 3.0; 5,000 ppm, 3.0; 15,000 ppm, 3.2). The increased severity of nephropathy in the 15,000 ppm male rats was accompanied by significantly increased incidences of mineralization of the blood vessels (4/52, 10/51, 6/51, 17/52) and glandular stomach (8/52, 13/51, 9/51, 23/52), parathyroid gland hyperplasia (14/51, 14/46, 12/49, 23/50), and fibrous osteodystrophy of the femur (12/52, 14/51, 12/51, 24/52). These lesions are manifestations of renal failure, uremia, and secondary hyperparathyroidism. The increased incidence of advanced renal disease caused reduced survival of the high-dose male rats.

No increase in the incidence of neoplasms in male or female rats was attributed to the ingestion of diets containing manganese (II) sulfate monohydrate.

2-YEAR STUDY IN MICE

Groups of 70 male and 70 female mice received diets containing 0, 1,500, 5,000, or 15,000 ppm manganese (II) sulfate monohydrate. These levels resulted in an average daily ingestion of 160, 540, or 1,800 mg/kg body weight (males) or 200, 700, or 2,250 mg/kg (females). Nine or 10 mice from each group were evaluated at the 9-month and 15-month interim evaluations.

Survival, Body Weights, Feed Consumption, and Clinical Findings

Survival rates of exposed male and female mice in the 2-year study were similar to those of the control groups. The mean body weights of exposed male mice were similar to that of the control group. Compared to controls, female mice had exposure-related lower mean body weights after week 37, and the final mean body weights for the 1,500, 5,000, and 15,000 ppm groups were 6%, 9%, and 13% lower than that of the control group. Feed consumption by all exposure groups was similar to that by the control groups. No clinical findings were attributed to the administration of manganese (II) sulfate monohydrate.

Hematology, Clinical Chemistry, and Tissue Metal Concentration Analyses

No chemical-related differences between exposed and control groups occured in hematology or clinical chemistry parameters. At the 9- and 15-month interim evaluations, tissue concentrations of manganese were significantly elevated in the livers of the 5,000 and 15,000 ppm groups. Hepatic iron levels were significantly lower in exposed females at the 9-month interim evaluation and in 5,000 and 15,000 males and all exposed females at the 15-month interim evaluation.

Pathology Findings

Incidences of thyroid follicular dilatation and hyperplasia were significantly greater in 15,000 ppm male and female mice than in controls. Follicular cell adenomas occurred in one 15,000 ppm male at the 15-month interim evaluation and in three 15,000 ppm males at the end of the study but not in the lower exposure groups or the control group. Follicular cell adenomas also occurred in two control, one 1,500, and five 15,000 ppm female mice at the end of the study. It is uncertain if the slightly increased incidence of follicular cell adenoma is related to the ingestion of manganese (II) sulfate monohydrate.

The incidences of focal hyperplasia of the forestomach epithelium were significantly greater in the 15,000 ppm male and exposed female groups. The hyperplasia was associated with ulcers and inflammation in some mice, particularly males.

GENETIC TOXICOLOGY

Manganese (II) sulfate monohydrate was not mutagenic in *Salmonella typhimurium* strains TA97, TA98, TA100, TA1535, or TA1537, with or without exogenous metabolic activation (S9), and did not induce sex-linked recessive lethal mutations in germ cells of male *Drosophila melanogaster*. Tests for induction of sister chromatid exchanges and chromosomal aberrations in cultured Chinese hamster ovary cells treated without S9 were positive; with S9, only the sister chromatid exchange test with manganese (II) sulfate monohydrate was positive.

CONCLUSIONS

Under the conditions of these 2-year feed studies, there was *no evidence of carcinogenic activity** of manganese (II) sulfate monohydrate in male or female F344/N rats receiving 1,500, 5,000, or 15,000 ppm. There was *equivocal evidence of carcinogenic activity* of manganese (II) sulfate monohydrate in male and female B6C3F_1 mice, based on the marginally increased incidences of thyroid gland follicular cell adenoma and the significantly increased incidences of follicular cell hyperplasia.

The ingestion of diets containing manganese (II) sulfate monohydrate was associated with an increased severity of nephropathy in male rats, focal squamous hyperplasia of the forestomach in male and female mice, and ulcers and inflammation of the forestomach in male mice. These studies were not designed to assess any neurotoxicity that might have been expected with chronic exposure to sufficiently high doses of manganese.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 9. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 11.

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Manganese (II) Sulfate Monohydrate

Male F344/N Rats	Female F344/N Rats	Male B6C3F$_1$ Mice	Female B6C3F$_1$ Mice
Doses			
0, 1,500, 5,000, or 15,000 ppm in feed (equivalent to 60, 200, or 615 mg/kg body weight per day)	0, 1,500, 5,000, or 15,000 ppm in feed (equivalent to 70, 230, or 715 mg/kg body weight per day)	0, 1,500, 5,000, or 15,000 ppm in feed (equivalent to 160, 540, or 1,800 mg/kg body weight per day)	0, 1,500, 5,000, or 15,000 ppm in feed (equivalent to 200, 700, or 2,250 mg/kg body weight per day)
Body weights			
15,000 ppm group lower than controls	Exposed groups similar to controls	Exposed groups similar to controls	Exposed groups lower than controls
2-Year survival rates			
25/52, 17/51, 21/51, 7/52	37/50, 37/50, 42/50, 36/48	45/50, 44/50, 46/51, 46/50	42/50, 46/50, 38/50, 42/51
Nonneoplastic effects			
Kidney: nephropathy severity (2.9, 3.0, 3.0, 3.2)	None	Thyroid gland: follicular cell focal hyperplasia (5/50, 2/49, 8/51, 27/50) Forestomach: focal squamous hyperplasia (2/50, 1/49, 5/51, 14/50); ulcer: (0/50, 0/49, 0/51, 6/50); inflammation: (0/50, 0/49, 0/51, 5/50)	Thyroid gland: follicular cell focal hyperplasia (3/50, 15/50, 27/49, 43/51) Forestomach: focal squamous hyperplasia (1/51, 3/50, 3/49, 9/50)
Uncertain effects			
None	None	Thyroid gland: follicular cell adenoma (0/50, 0/49, 0/51, 3/50)	Thyroid gland: follicular cell adenoma (2/50, 1/50, 0/49, 5/51)
Level of evidence of carcinogenic activity			
No evidence	No evidence	Equivocal evidence	Equivocal evidence
Genetic toxicology			
Salmonella typhimurium gene mutation:		Negative in strains TA97, TA98, TA100, TA1535, and TA1537 with and without S9	
Sister chromatid exchanges Cultured Chinese hamster ovary cells *in vitro*:		Positive with and without S9	
Chromosomal aberrations Cultured Chinese hamster ovary cells *in vitro*:		Positive without S9; negative with S9	
Sex-linked recessive lethal mutations *Drosophila melanogaster*:		Negative when administered in feed or by injection	